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# **Brief Technical Note**

# A NEW DEVICE TO HELP FACILITATE MANUAL SPERMATOPHORE TRANSFER IN PENAEID SHRIMP

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#### ABSTRACT

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A continuous gill irrigator has been developed, in combination with a restraining device, to keep female penaeid shrimp alive and to reduce stress during manual spermatophore transfer (a form of artificial insemination). Construction, cost, and use of the equipment are described.

#### INTRODUCTION

Shrimp culture is progressing to the point where genetic programs can begin to help increase production. In scientifically sound breeding programs it is often necessary to produce and identify full-sib families and/or half-sib families and to identify all parents. The current spawning techniques of placing many shrimp in large tanks is not adequate for such work.

One technique which will allow the production and identification of such families is manual spermatophore transfer (a form of artificial insemination). By extracting a spermatophore and manually placing it on or in the thelycum of the female, it is easy to identify both parents and progeny. This technique may be necessary when producing intra- and inter-specific hybrids because behavioral and thelyca structural differences among strains and species can serve as pre-fertilization barriers. Manual spermatophore transfer can circumvent these problems and may enable shrimp breeders to produce valuable hybrids.

One of the biggest problems in spermatophore transfer is the amount of time needed to position the spermatophore properly. Shrimp are easily stressed by short periods out of water, and early attempts at spermatophore transfer were largely unsuccessful because the females were stressed and either died, were unable to spawn, or released only a few thousand eggs, the quality of which was suspect.

To circumvent this problem, a continuous gill irrigator has been developed, in conjunction with a restraining device, to allow gas exchange during spermatophore transfer and thus reduce stress.

## MATERIALS AND METHODS

The gill irrigator (Fig.1) is constructed from a separatory funnel, which serves as the water reservoir, a glass Y-tube, heated and bent to the shape shown in Fig.1, and two 0.5-ml disposable glass pipettes, the tips of which have been heated and bent 90° to direct water flow in a horizontal direction. The pieces of glass are joined by short sections of rubber tubing. The size and

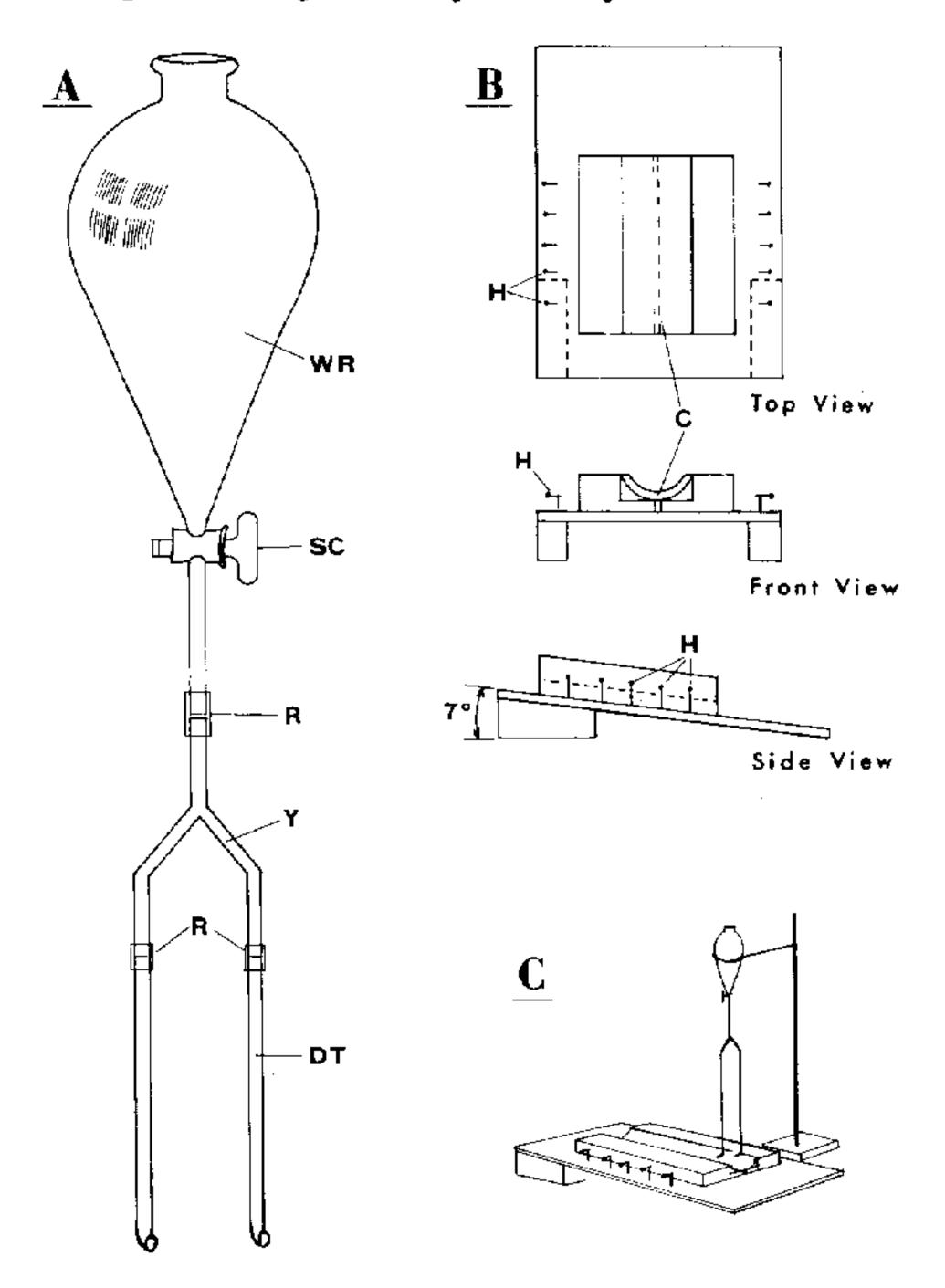


Fig.1. Gill irrigator and restraining device used during spermatophore transfer. A. The gill irrigator: WR, water reservoir; SC, stopcock; R, rubber tubing; Y, Y-tube; DT, delivery tubes. B. Restraining device: H, hooks; C, cradle. C. Overall view of the gill irrigator and restraining device positioned as it is used during spermatophore transfer. A ring stand is used to support the gill irrigator.

shape of the water reservoir are unimportant, but a separatory funnel is advantageous in that it has a stopcock which can be used to regulate water flow.

The restraining device (cradle) is a 20-cm length of 3-cm P.V.C. pipe cut in half along its length and glued to a piece of wood. The wood is angled at 7° to prevent water from running over the thelycum during spermatophore transfer. Five pairs of hooks are set in two parallel rows, one on each side of the cradle, to hold a piece of Vexar¹ screen that is used to restrain the female while she is in the cradle. A small hole was cut in the Vexar screen so that the thelycum would be exposed during spermatophore transfer. Rubber bands were used to restrain the female during early trials; however, the Vexar screen is far more effective in restraining the female. Moreover, the rubber bands injured several females.

The female that will receive the spermatophore is placed in the cradle, ventral side up, and securely positioned with the Vexar screen. The anterior end of the shrimp is placed in the lower end of the cradle to prevent water from flowing over the posterior portions of the shrimp during spermatophore transfer. The delivery tubes are placed in each branchial cavity, and a continuous flow of water bathes each set of gills. The direction of the water flow does not appear to be important. Both anteriorly and posteriorly directed water have been effective.

Total cost for both apparatuses is approximately U.S. \$22, and they can be assembled within an hour.

### DISCUSSION

Since the gill irrigator has been used, no female has died or gone into stress during spermatophore transfer; females have been kept on the irrigator for ten minutes and have resumed normal behavior within a minute of their return to water. Flow rates of between 40 and 60 ml/min have been adequate.

The use of this apparatus has enabled us to have some success with manual spermatophore transfer. Eighty-eight percent of the females that have received spermatophores have spawned. Spawns have ranged from 38,000 to 212,000 eggs, and some larvae have been raised through protozoea I. More research is needed to refine this technique further so that it is predictable and reliable. The use of the gill irrigator allows more precise spermatophore transfer than would otherwise be possible. That, coupled with the fact that the gill irrigator reduces stress during the female's time out of water, should allow the future refinement of this technique and should increase its success rate.

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<sup>&</sup>lt;sup>1</sup> The use of this trade name does not imply endorsement by the federal government.